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Investigating the Effects of a Cardiotoxic Drug on Calcium Homeostasis in the Heart
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Contractile dysfunction and arrhythmogenic activity can arise if there are perturbations to calcium (Ca) homeostasis in the heart, such as in response to cardiotoxic substances. This investigation focuses on changes in cellular Ca homeostasis in response to the acute application of the cardiotoxic drug Clozapine (an atypical anti-psychotic).
Rat ventricular myocytes were isolated and stimulated under voltage clamp control. Various parameters of Ca handling were measured both in control conditions and in the presence of 10 μM clozapine. Ca-sensitive indicators fluo3-AM or fura-2 were used to quantify cytosolic Ca. Sarcoplasmic reticulum (SR) Ca content was measured by applying 10 mM caffeine. Activity of Ca extrusion mechanisms (SERCA - sarco-endoplasmic reticulum ATPase, NCX - sodium-calcium exchanger) were calculated from Ca transient decay rates. The current-voltage (I-V) relationship of the L-type Ca channel was also measured.
Acute application of clozapine significantly reduced mean Ca transient amplitude by 48% and L-type Ca current (ICa_L) density by 50%. SR content was decreased by 18 % and SERCA activity (rate constant) was reduced by 34 %. NCX rate constant was reduced by 17%. The effect of clozapine on SERCA was also present in phospholamban knockout myocytes suggesting that the effect on SERCA is not dependent on phospholamban.
It remains unclear if or how the observed effects on Ca handling contribute to the cardiotoxicity of clozapine observed in the clinical setting (arrhythmogenesis and myocarditis). We speculate that effects on ICa_L may predispose to refractory heterogeneity and reduced SERCA activity may increase the chance for Ca wave propagation. Current work focuses on effects of clozapine on calcium spark activity and SR threshold for Ca waves.

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Redox Modification of Ryanodine Receptors underlies Abnormal Calcium Handling in Aging Rabbit Hearts
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Aging alters the electrical activity of the heart and provides a pro-arrhythmic substrate that increases the risk of arrhythmia and sudden cardiac death (Cooper *et al.* 2012). Although it is known that aberrant calcium dynamics may be a source of electrical abnormalities, the underlying mechanisms that affect age-associated alterations in triggered activity remain unclear. In the present study, we used ventricular myocytes isolated from young (5-9 months) and old (4-5 years) rabbit hearts to test the hypothesis that changes in Ca^{2+} homeostasis are caused by post-translational modification of ryanodine receptors (RyRs) caused by increased production of reactive oxygen species (ROS) in the aging heart. Changes in parameters of Ca^{2+} handling were determined by measuring cytosolic and intra-sarcoplasmic reticulum (SR) Ca^{2+} dynamics in intact and permeabilized ventricular myocytes using confocal microscopy. Aging did not change SERCA activity and spark frequency but decreased spark amplitude and SR Ca^{2+} content in permeabilized cells, suggesting an increased activity of RyRs. Under stimulation withβ-adrenergic agonist isoproterenol, Ca^{2+} transient amplitude, SR Ca^{2+} load, and latency of pro-arrhythmic spontaneous Ca^{2+} waves (SCWs) were decreased in cardiomyocytes derived from old rabbits; whereas, the rate of ROS production was increased. Treatment with the antioxidant DTT attenuated RyR-mediated SR Ca leak in aged permeabilized myocytes to levels comparable to young. Moreover, pretreatment with the mitochondria-specific ROS scavenger mito-TEMPO ablated SCWs in isoproterenol-treated old cardiomyocytes to levels comparable to young. This data suggests that increased rate of ROS production by mitochondria lead to the thiol-oxidation of RyRs, which underlies the hyperactivity of RyRs and thereby shortened refractoriness of Ca^{2+} release in cardiomyocytes from the aging heart. This mechanism likely plays an important role in the increased risk of arrhythmia and sudden cardiac death in the aging population.

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Dantrolene Prevents Spontaneous Calcium Waves in Cardiac Myocytes from Aged Mice
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Contractile dysfunction in the aged heart reflects abnormalities in intracellular cycling of Ca through the sarcoplasmic reticulum (SR). Dantrolene exerts antiarrhythmic effects on cardiac myocytes from animal models of heart failure, presumably by preventing spontaneous diastolic Ca release through ryanodine receptor channels. In this study we tested the hypothesis that dantrolene prevents diastolic Ca release in the Aged myocardium. Left-ventricular myocytes isolated from Young (3-4 month) and Aged (24-26 month) C57BL/6 male mice were loaded with the Ca indicator fluo-4/AM and exposed to elevated extracellular Ca (10 mM) to induce spontaneous Ca waves. Ca waves were observed at similar frequencies in Aged (10.2 ± 1.7 waves/min) and Young (8.7 ± 2.7 waves/min), yet waves in Aged were of smaller amplitude (F/Fo = 3.9 ± 0.5 Aged versus 6.0 ± 0.3 Young, $P<0.05$) and occurred at a lower threshold SR Ca content as assessed by application of 10 mM caffeine (F/Fo,Caffeine= 4.8 ± 0.4 Aged versus 7.4 ± 0.2 Young, $P<0.05$). Dantrolene treatment (1 μM, 5 min) in Aged decreased wave frequency (to 7.3 ± 1.7 waves/min, $P<0.05$), increased wave amplitude (to F/Fo= 5.0 ± 0.5, $P<0.05$), and increased threshold SR Ca content (to F/Fo,Caffeine= 6.1 ± 0.4, $P<0.05$) but had no effect in Young. Dantrolene had no effect on the amplitude of action-potential induced Ca transients (0.5 Hz, 2 mM extracellular Ca) in Aged or Young. Thus, dantrolene can have beneficial effects on Ca cycling in Aged cardiomyocytes which may prove useful in treating cardiac dysfunction commonly associated with advancing age. (Support: NIH K01AG041208, RO1HL086483).

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An Assessment of Calcium Handling and Cardiovascular Drug-Profiling in Cytiva Embryonic Stem-Cell Derived Cardiomyocytes
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There is momentum towards using stem cell-derived cardiomyocytes (SCCM) to assess the safety and efficacy of cardiovascular (CV) drugs in early-phase development. However, issues of phenotypic heterogeneity and functional maturation impact on the utility of SCCM in CV drug development pathways. We used confocal imaging and our SALVO framework to profile spontaneous and field-stimulated Ca^{2+} handling and oscillatory behaviour in GE Healthcare's SCCM (Cytiva^{TM}). Cytiva were supplied as a heterogeneous cell population (47.5 ± 13.7% CMs, n=5 batches) and the proportion of cells that exhibited spontaneous Ca^{2+} oscillations correlated with cardiac troponin-T (cTNT) positive staining (CMs). In heterogeneous populations spontaneous Ca^{2+} oscillations in CMs exhibited significant batch-to-batch variability and were predominantly characterised by slow release and prolonged decay kinetics. Intercellular synchronization of Ca^{2+} release did not require physical cell-to-cell contact and was independent of the plating density and the local non-CM population. The amplitude and temporal patterning of spontaneous Ca^{2+} release in CMs became progressively disorganized beyond day 7 and we did not observe functional maturation of CMs in culture over 21 days. Ca^{2+} handling in those CMs exhibiting spontaneous oscillations could be positively modulated by field stimulation which improved amplitude and kinetic parameters of Ca^{2+} release and decay. Enrichment of CMs (>1.8-fold) using affinity methods directed at the cell-surface signal regulatory protein alpha (SIRPa) improved spontaneous Ca^{2+} handling possibly indicating a negative role of non-CMs in the heterogeneous population. Data will be presented on the concentration-response profiling of experimental and clinically-licensed cardioactive drugs using these SCCMs.

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Mitochondria-Derived Ros Disturb Ca^{2+} Cycling and Evoke Abnormal Action Potentials in Guinea-Pig Ventricular Myocytes: A Theoretical Study
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Abnormal Ca^{2+} handling often results in contractile dysfunction and ventricular arrhythmias, the leading causes of sudden cardiac death. However, in spite of extensive efforts, the molecular mechanisms underlying disturbance of Ca^{2+} cycling are not completely understood, hindering the development of effective